Frequent Genetic Heterogeneity in the Clonal Evolution of Gynecological Carcinosarcoma and Its Influence on Phenotypic Diversity

Hiroaki Fujii, Manabu Yoshida, Zhu Xue Gong, Toshiharu Matsumoto, Yoshitomo Hamano, Masaharu Fukunaga, Ralph H. Hruban, Edward Gabrielson, and Toshikazu Shirai

Departments of Pathology II [H. F., Z. X. G., Y. H., T. S.], Pathology I [T. M.], and Obstetrics/Gynecology [M. Y.], Juntendo University School of Medicine, Tokyo 113-8421, Japan; Department of Pathology I, Jikei University School of Medicine, Tokyo, Japan [M. F.]; and Departments of Pathology [E. G., R. H. H.] and Oncology Center [E. G.], Johns Hopkins University School of Medicine, Baltimore, Maryland 21287

ABSTRACT

Carcinosarcomas of the uterus, ovaries, and fallopian tubes are highly aggressive neoplasms with incompletely understood histogenesis. Although recent immunohistochemical, cell culture, and molecular genetic studies have favored these cancers to be monoclonal in origin, the extent of intratumoral genetic heterogeneity in these tumors with divergent histology has not been reported previously. For this study, we microdissected a total of 172 carcinomatous or sarcomatous foci from 17 gynecological carcinosarcomas and analyzed allelic status with 41 microsatellite markers on chromosomal arms 1p, 1q, 3p, 4q, 5q, 6q, 8p, 9p, 10q, 11p, 11q, 13q, 16q, 17p, 17q, 18q, and 22q. With the exception of a single case with microsatellite instability, we found shared allelic losses and reten-

INTRODUCTION

CSs3 of the female genital tract are uncommon but clinically highly aggressive neoplasms with biphasic histology of carcinomatous and sarcomatous elements (1–3). These neoplasms are usually large and bulky tumors and have often spread beyond the primary organ at the time of initial surgery (3–5). Histologically, the carcinomatous components can include endometrioid, clear cell, serous, squamous, or mucinous differentiation, or they may be undifferentiated. Sarcomatous components include homologous (endometrial stromal sarcoma, fibrosarcoma, and leiomyosarcoma) elements and, in up to 50% of cases, heterologous (chondrosarcoma, osteosarcoma, rhabdomyosarcoma, and liposarcoma) elements.

Because these tumors have an intimate admixture of histologically divergent malignant cells, the histogenesis of CSs has long been a matter of speculation and dispute (2, 3, 6, 7). Three main hypotheses exist to explain these diverse histological elements. In the “collision tumor theory,” synchronous bicolonal tumors are thought to blend together to form CS histology. In the “composition theory,” the stromal element is thought to be reactive and not neoplastic. This second theory has largely been abandoned because metastatic foci also frequently contain malignant stromal elements as well as malignant epithelial elements. The third, and most favored theory, is the “combination tumor theory,” which postulates that both elements originate from a single stem cell clone. According to this theory, the sarcomatous component arises in a carcinoma through evolution of subclones, and thus the carcinomatous component is the primary determinant of their behavior and prognosis (8, 9).

A number of recent cell culture, immunohistochemistry, and molecular genetic studies support the monoclonal nature of these neoplasms. For example, cell lines established from CSs have been shown to differentiate into epithelial, mesenchymal, or both components under various culture conditions (10–13). Furthermore, immunohistochemical studies have documented the expression of epithelial markers in the sarcomatous components of a large proportion of cases (13–19). More recently, X-chromosomal inactivation assays, p53 mutational analyses, and LOH studies have all shown the carcinomatous and sarcomatous elements to share common genetic alterations (20–24).

Although these recent studies provide convincing evidence to support the monoclonal origin of these cancers, genetic diversity corresponding to phenotypic diversity has not been reported previously. It is possible to follow the clonal evolution of cancers with markers to identify LOH. In our previous studies of breast cancer progression, we evaluated LOH on multiple chromosomal regions in multiple individually microdissected foci of ductal carcinoma in situ and found patterns of LOH consistent with genetic progression and heterogeneity superimposed on homogeneous genetic changes (25–26). For this study, we have used a similar approach to evaluate the genetic evolution of gynecological CSs. Loss of specific alleles at 17 chromosomal loci suspected to harbor tumor suppressor genes was characterized using microsatellite markers in multiple individually microdissected foci of these cancers, and by comparing LOH patterns, we deduced the likely order of genetic changes in the evolution of individual tumors.

MATERIALS AND METHODS

Tissues. Cases of gynecological CS were identified in the archival pathology files of Juntendo University and Jikei University, confirmed by review of histopathology and tested for the quality of DNA preservation. Of the 17 cases identified, 12 cases originated in the uterus, 3 cases originated in the ovary, and 2 in the fallopian tube (summarized in Table 1). Heterologous elements were identified in 14 cases, and the remaining 3 cases contained only homologous elements. Coexistence of other gynecological tumors was identified in case CS2 (CS in the uterus, endometrioid carcinoma in the bilateral ovaries), case CS9 (CS in the uterus and endometrioid carcinoma in the left ovary), and case CS21 (CS in the left fallopian tube and endometrioid carcinoma in the ovary and the uterus).

Microdissection and DNA Extraction. Serial 8-μm sections were cut, deparaffinized, stained with H&E, visualized with an inverted microscope, and microdissected using a 26-gauge needle. Three to 34 carcinomatous and

Received 5/21/99; accepted 10/28/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by a research grant from the Ministry of Education, Science, Sports and Culture of Japan.

2 To whom requests for reprints should be addressed, at Department of Pathology II, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Phone: 03-5802-1039; Fax: 03-3813-3164; E-mail: hfujii@med.juntendo.ac.jp.

3 The abbreviations used are: CS, carcinosarcoma; LOH, loss of heterozygosity.
Table 1  Summary of carcinosarcoma cases and their genetic changes

For each case, location, histology (homologous or heterologous), number of tumor foci dissected, results for microsatellite analysis, and deduced genetic patterns are presented.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histology</th>
<th>Dissected foci</th>
<th>Uniform LOH throughout(^a)</th>
<th>Heterogeneous LOH(^b)</th>
<th>Genetic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS1</td>
<td>Left ovary, metastasis tolung, liver, retroperitoneum, lymph nodes, and others</td>
<td>Heterologous</td>
<td>34</td>
<td>10q and 11q (metastatic CS foci, lung)</td>
<td>Homogeneous in ovarian C/S, Lung metastasis with genetic progression C/S</td>
</tr>
<tr>
<td>CS2</td>
<td>Uterus (CS), bilateral ovaries (endometrioid carcinoma)</td>
<td>Heterologous</td>
<td>12</td>
<td>MIa MIb in uterus, No MI in ovary</td>
<td>Mutator phenotype, collision in the uterus, Ovary third primary</td>
</tr>
<tr>
<td>CS3</td>
<td>Uterus</td>
<td>Heterologous</td>
<td>15</td>
<td>1, 9p, 10q, 16q, 17q</td>
<td>Genetic progression and diversion in both carcinoma/sarcoma foci</td>
</tr>
<tr>
<td>CS4</td>
<td>Uterus</td>
<td>Heterologous</td>
<td>11</td>
<td>3p, 6q, 18q</td>
<td>CS with diversion, in some of the carcinoma and sarcoma foci</td>
</tr>
<tr>
<td>CS5</td>
<td>Uterus</td>
<td>Heterologous</td>
<td>10</td>
<td>9p, 16q, 17p</td>
<td>CS with diversion, in some of the carcinoma and sarcoma foci</td>
</tr>
<tr>
<td>CS6</td>
<td>Ovary, invasion to uterine wall</td>
<td>Homologous</td>
<td>4</td>
<td>9p, 11q, 17p</td>
<td>Genetic progression in some of the carcinoma foci</td>
</tr>
<tr>
<td>CS7</td>
<td>Uterus, invasion to abdomen and ovary</td>
<td>Heterologous</td>
<td>11</td>
<td>9p</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>CS8</td>
<td>Bilateral ovaries, invasion to peritoneum</td>
<td>Heterologous</td>
<td>11</td>
<td>3p, 11q, 13q, 16q, 17p</td>
<td>Homogeneous CS with metastatic foci</td>
</tr>
<tr>
<td>CS9</td>
<td>Uterus (CS) and left ovary (endometrioid carcinoma)</td>
<td>Heterologous</td>
<td>11</td>
<td>Uterine CS: 1p(^a), 4q, 6q(^a), 13q, Ovary: 1p(^b), 6q(^b)</td>
<td>Homogeneous CS: ovary: second primary</td>
</tr>
<tr>
<td>CS11</td>
<td>Uterus</td>
<td>Heterologous</td>
<td>3</td>
<td>3p, 6q, 8q, 9p, 13q, 18q</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>CS13</td>
<td>Uterus, invasion to peritoneum</td>
<td>Heterologous</td>
<td>6</td>
<td>4q, 5q, 8p, 17q, 22q</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>CS14</td>
<td>Uterus</td>
<td>Homologous</td>
<td>7</td>
<td>9p, 22q</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>CS17</td>
<td>Fallopian tube</td>
<td>Heterologous</td>
<td>7</td>
<td>3p, 4q, 8p, 9p, 11p, 17p, 17q</td>
<td>Genetic progression in some of the carcinoma foci</td>
</tr>
<tr>
<td>CS18</td>
<td>Fallopian tube</td>
<td>Heterologous</td>
<td>7</td>
<td>1q, 13q, 17p</td>
<td>CS with genetic progression in some of the sarcoma foci</td>
</tr>
<tr>
<td>CS19</td>
<td>Uterus</td>
<td>Heterologous</td>
<td>7</td>
<td>16q, 17p, 17q</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>CS20</td>
<td>Fallopian tube (CS), ovary (canceroma), uterus (canceroma)</td>
<td>Homologous</td>
<td>5</td>
<td>1, 4q, 9p, 17p, 18q, 22q</td>
<td>Endometrial carcinoma, with genetic progression to metastatic CS</td>
</tr>
<tr>
<td>CS21</td>
<td>Fallopian tube (CS), ovary (canceroma), uterus (canceroma)</td>
<td>Homologous</td>
<td>11</td>
<td>6q, 17p</td>
<td>1, 10q (T2–T6; Fallopian tube-CS)</td>
</tr>
</tbody>
</table>

Total 172

\(^a\) Chromosomal arms on which all of the dissected foci showed homogeneous allelic losses.

\(^b\) Chromosomal arms on which only a portion of the dissected foci (C, carcinoma foci; S, sarcoma foci, CS, both carcinoma and sarcoma foci) showed allelic losses; MI, microsatellite instability, pattern a and b.
sarcomatous foci were individually microdissected from each neoplasm (Fig. 1). In case CS1 and case CS21, metastatic foci were also microdissected. A total of 172 foci were microdissected from the 17 cases. Normal control tissue was also dissected from the adjacent nonmalignant stroma, epithelium, or inflammatory infiltrates. Microdissected tissue was digested overnight at 50°C in buffer containing 0.5% NP40, 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 200 μg/ml proteinase K. The lysate was heated at 95°C for 10 min and was stored at −20°C until used directly in PCR reaction.

Detection of LOH. PCR reactions contained 1 μl of DNA lysate, 0.4 μM[γ-32p]ATP radiolabeled microsatellite primer, 0.2 mM deoxynucleotide triphosphate, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, and 0.4 unit of Taq polymerase in a total reaction volume of 10 μl. Taq was added to reactions prewarmed to 94°C (hot start PCR), and samples were amplified with 35 cycles of PCR amplification. PCR products were separated on a 5% denaturing polyacrylamide-urea-formamide gel, and LOH was determined by more than a 75% reduction of the intensity in one of the two alleles as compared with those in normal control. When only a portion of the foci dissected from a single CS showed LOH, PCR reactions were repeated in duplicate to confirm the LOH and to exclude spurious PCR reactions. If necessary, microdissection was repeated.

All of the PCR primers for microsatellite markers were purchased from Research Genetics Co., Ltd. (Huntsville, AL). Microsatellite markers were selected to cover commonly deleted chromosomal regions in many of human tumors. The following primers were used: 1p and 1q (D1S500, D1S228, D1S58, and D1S188), 3p (D3S1296 and D3S1293), 4q (D4S424, D4S415, and D4S413), 5q (D5S2072, D5S221, and D5S1956), 6q (D6S264, D6S473, and D6S255), 8p (D8S255, D8S264, and D8S261), 9p (D9S1748 and D9S1749), 10q (D10S221, D10S219, and D10S574), 11q (D11S124), 11q (D11S29 and Im2), 13q (D13S166 and D13S171), 16q (D16S265, D16S541, and D16S261), 17p (TP53, CHRNB1, and D17S786), 17q (D17S588 and D17S579), 18q (D18S46, D18S55, D18S474, and D18S487), and 22q (D22S270).

RESULTS

One case, CS2, was exceptional in showing novel microsatellite alleles (microsatellite instability). All other tumors showed LOH homogeneously throughout the lesions at one to eight chromosomal loci (Table 1). The highest frequency of homogeneous LOH was seen on 17p (11 cases, 64.7%), followed by 9p (7 cases, 41.2%), 13q (6 cases, 35.3%), 4q (5 cases, 29.4%), 3p, 6q, 8p, and 18q (4 cases, 23.5%), 11q, 16q, 17q, and 22q (3 cases, 17.6%), chromosome 1 and 5q, (2 cases, 11.8%), 11p (1 case, 5.9%), and 10q (0 cases). On average, 3.65 chromosomal arms showed LOH.

In eight of these cases, all tumor foci examined showed identical patterns of loss or retention at all alleles tested. Although each of these tumors had a distinctive bidirectional phenotype, no genetic heterogeneity could be detected.

In contrast to these neoplasms with homogeneous LOH, heteroge-
neous patterns of LOH were noted among individually microdissected foci in eight cases (cases CS1, CS3, CS4, CS5, CS6, CS18, CS19, and CS21). Heterogeneous LOH occurred with one of the following patterns: (a) identical LOH at some loci (consistent with monoclonal origin) and identical additional alleles lost in both carcinoma foci and sarcoma foci of metastatic tumor (one case, CS1; Fig. 2); (b) additional LOH consistent with genetic progression in some of the sarcoma foci (one case, CS19; Fig. 3); (c) additional LOH consistent with genetic progression in some of the carcinoma foci (two cases, CS6 and CS18); (d) differing LOH consistent with genetic diversion in some of the carcinoma and sarcoma foci (two cases, CS4 and CS5); (e) additional LOH consistent with genetic progression from original carcinoma to CS only in metastatic foci (one case, CS21; Fig. 4); and (f) additional LOH consistent with genetic progression and diversion from original carcinoma to carcinoma/sarcoma foci (one case, CS3; Fig. 5).

In addition to these complex patterns of genetic progression and diversion, one case (CS2) was exceptional in that extensive microsatellite instability of most of the microsatellite markers was noted in both carcinomatous and sarcomatous foci. Although many similar new alleles were identified within carcinoma foci or sarcoma foci independently, only a few of the new alleles were shared between carcinoma and sarcoma foci. This strongly suggested the biclonal nature of the uterine CS lesion in this case. An ovarian endometrioid carcinoma occurring in the same individual did not show any of these allelic changes and thus most likely represents a third primary tumor.

Patterns of chromosomal loss (i.e., homogeneous or heterogeneous) in the different neoplastic components of the CSs are summarized in Fig. 6. Loss of several chromosomal arms (17p, 3p, 4q, 6q, 9p, 13q, and 22q) is relatively homogeneous throughout different regions of the tumors. These homogeneous patterns of LOH suggest that these genetic changes occurred relatively early in the clonal evolution of these neoplasms. Conversely, other chromosomal losses (10q, 11p, 11q, 1p, and 1q) were commonly seen in some but not all areas of the neoplasms. These heterogeneous patterns of LOH suggest that these genetic alterations occurred relatively late in the development of the neoplasms. Notably, LOH of 10q was found in only metastases of two cases. The possibility that inactivation of PTEN or another gene on 10q contributes to metastases of these cancers warrants further investigation.

**DISCUSSION**

Although cancers have long been recognized to consist of clonal populations of cells, only recently have some neoplastic lesions have been shown to have genetically heterogeneous elements (25–32). In many situations, genetic heterogeneity occurs in tumors that have a relatively homogeneous histological appearance. For example, we recently described substantial genetic heterogeneity in early *in situ* carcinoma of the breast.

It is reasonable to question whether genetic heterogeneity can account for the phenotypic divergence of CSs, which have been shown previously to originate from a single clone. Since the first description by Virchow (8, 44), CSs have been reported in ever-increasing number of locations in the human body, such as gastrointestinal tract, upper aerodigestive tract, lung, breast, and genitourinary tract (8, 33–37). To undertake our study of gynecological CSs, we examined numerous individually microdissected foci of both carcinomatous and sarcomatous cells for LOH using microsatellite markers. Sixteen of the 17 cases we studied showed allelic losses shared by
all tumor foci, consistent with a monoclonal origin for these tumors. In eight of the cases, all of the dissected foci shared homogeneous genetic changes in both carcinomatous and sarcomatous areas, and no discordant losses were identified. These findings strongly indicate the totipotential nature of the single tumor clone and hence support the combination theory of CS histogenesis.

In addition to this extensive sharing of chromosomal changes, we also noted evidence for genetic progression or genetic diversion within eight of the tumors. In these tumors, we found LOH of additional chromosomal arms in some of the dissected foci (progression) or differing patterns of LOH of one or more chromosomal arms (diversion). Summarizing the pattern of genetic changes observed in
our cases, we hypothesize several possible genetic and phenotypic relationships in the clonal evolution of gynecological CSs as shown in Fig. 7. For each tumor, there appears to be a totipotential clone from which all other portions of the tumor evolve. An original clone of a pure carcinoma can then apparently acquire carcinosarcomatous or sarcomatous phenotype by successive genetic changes (CS3 and CS21). On the other hand, we saw no evidence for tumors in which a sarcoma appeared to give rise to carcinosarcomatous or carcinomatous subclones.

The prognosis of CSs has been proposed to be related to characteristics of specific histological elements, but there is no agreement concerning which histological elements determine the prognosis of these tumors. Sarcomatous components, and especially the presence of rhabdomyosarcomatous components, have been associated with poor outcome, but a number of studies have also emphasized the carcinomatous component as the primary determinant of prognosis (2, 4, 9, 17, 38, 39). Our data demonstrate both carcinomatous and sarcomatous foci to independently have the ability to acquire advanced genetic changes during the evolution of the tumor. It appears reasonable, therefore, that both components of the tumor should be considered when attempting to assess the malignant potential of a particular tumor.

Overall, the high percentage of loci with LOH in these tumors and the high frequency of genetic heterogeneity among different portions...
of tumors may be related to the relatively aggressive behavior of these cancers. We found the highest frequency of LOH on 17p, a chromosomal arm that includes the p53 locus, and frequent p53 mutations and overexpression have been reported previously in these neoplasms (19, 20, 22–24, 40). Because p53 overexpression and 17p LOH both occur concordantly in sarcomatous and carcinomatous components, we suspect that p53 is affected very early in the development of the majority of these tumors. Somewhat surprisingly, LOH of 10q was only infrequently noted in CSs. This chromosomal arm harbors the recently identified tumor suppressor PTEN, which is frequently mutated in endometrioid carcinoma (41–43).

The results of our study raise the possibility that inactivation of PTEN, commonly seen in usual endometrial carcinoma, is a relatively uncommon event in the highly malignant CSs. LOH at the PTEN locus on 10q, when it does occur in CSs, may occur late in the progression of these neoplasms. In contrast, inactivation of p53 (and LOH of 17p) appear to be frequent in the pathogenesis of CSs, similar to what is seen in aggressive serous variants of endometrial adenocarcinoma. The possibility that PTEN and p53 alterations are significantly different prognostic implications in developing gynecological neoplasms warrants further investigation in larger samples of these cancers. Together, these findings suggest that CSs are genetically distinct from the majority of endometrial cancers.

In conclusion, our data support the concept that gynecological CSs are in most cases derived from a single clone. The extensive sharing of many genetic alterations by phenotypically diverse areas of tumors suggests that the phenotypic divergence occurs relatively late in the evolution of the cancers. Furthermore, we have demonstrated genetic heterogeneity to accompany the phenotypic divergence, with patterns of genetic alterations that are consistent with both progression and divergence within individual tumors.

REFERENCES

17. Mayall, F., Rutty, K., Campbell, F., and Goddard, H. P53 immunostaining suggests that the phenotypic divergence occurs relatively late in the development of the majority of these tumors. Somewhat surprisingly, LOH of 10q was only infrequently noted in CSs. This chromosomal arm harbors the recently identified tumor suppressor PTEN, which is frequently mutated in endometrioid carcinoma (41–43).

REFERENCES

Frequent Genetic Heterogeneity in the Clonal Evolution of Gynecological Carcinosarcoma and Its Influence on Phenotypic Diversity

Hiroaki Fujii, Manabu Yoshida, Zhu Xue Gong, et al.

*Cancer Res* 2000;60:114-120.

Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/60/1/114

Cited articles This article cites 40 articles, 8 of which you can access for free at: http://cancerres.aacrjournals.org/content/60/1/114.full.html#ref-list-1

Citing articles This article has been cited by 6 HighWire-hosted articles. Access the articles at: /content/60/1/114.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.